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LETTER

Redox regulation of neurovascular function by acetazolamide: complementary insight into mechanisms underlying high-altitude acclimatisation

We read with interest the study by Fan and colleagues describing how the carbonic anhydrase (CA) inhibitor acetazolamide reduced periodic breathing and accelerated acclimatisation to high altitude in native lowlanders (Fan *et al.* 2012). Intravenous infusion was shown to increase resting cerebral blood flow (CBF) and cerebrovascular reactivity to carbon dioxide (CVR_{CO_2}) independently of altered peripheral or central chemoreflex sensitivity. The authors concluded that the improvement in breathing stability was attributable to an increase in the 'CO_2 reserve'. We would like to extend their elegant findings by suggesting a complementary mechanism albeit secondary to classical CA inhibition that may have equally contributed to the neurovascular benefits as observed.

A closer look at the molecular structure of acetazolamide (5-acetamido-1,3,4-thiadiazole-2-sulfonamide) reveals a compound with clear 'antioxidant potential'. The thiadiazole moiety is a heterocyclic compound that consists of one sulphur and two nitrogen atoms housed within an aromatic five-membered ring (Fig. 1). Serving not only as a constrained pharmacophore, this moiety can also serve as a hydrogen-binding domain and two-electron donor system. Numerous derivatives have since been synthesised and shown to exhibit potent radio-protective, anti-microbial, anti-inflammatory and antioxidant properties. Indeed, thiol derivatives of thiadiazoles have been shown to act even more effectively as antioxidants with the radical being 'reduced' by the thiol group and subsequently 'trapped' through electron delocalisation within the aromatic ring (Cressier *et al.* 2009). *In vitro* and theoretical approaches have further identified these compounds to be targeted scavengers of the hydroxyl radical (HO•) (Prouillac *et al.* 2009), a species with unrivalled molecular reactivity and thermodynamic capacity to cause indiscriminate cell damage. Indeed, investigators regularly exploit decarboxylation and hydroxylation reactions of aromatic compounds to facilitate molecular detection of HO• *in vivo*. Ironically, however, such an affinity for free radicals underlies acetazolamide's photoxicity (Vargas *et al.* 1998).

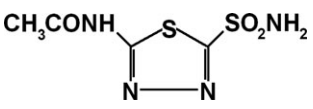

Figure 1. Molecular structure of acetazolamide

Why should targeted HO• scavenging benefit acclimatisation to high altitude? Human studies have taken advantage of electron paramagnetic resonance spin trapping and shown hypoxia to increase the systemic, pulmonary and cerebral formation of free radicals, with further increases known to occur with exercise, stressors that are unified by their ability to liberate catalytic iron (Bailey *et al.* 2009). Indeed, this technique has facilitated targeted detection of HO• in the cerebrospinal fluid of humans exposed to hypoxia (Bailey *et al.* 2009). *In vitro* studies indicate that this may be due to a hypoxia-mediated increase in the mitochondrial formation of the superoxide anion which has the potential to form downstream HO• subsequent to Fenton and Haber–Weiss chemistry. Though biologically important in physiologically controlled, though as of yet, undefined amounts, these radicals have equal potential to cause membrane damage, scavenge nitric oxide (NO) and impair vascular endothelial function when in excess (Bailey *et al.* 2009). Targeted scavenging via oral antioxidant prophylaxis has since been shown to benefit acclimatisation by improving systemic oxygenation and reducing the neurological symptoms associated with acute mountain sickness (Bailey & Davies, 2001).

Thus, could intravenous acetazolamide infusion serve to reduce free radical formation, liberate NO and thus improve neurovascular function at high altitude? This alternative mechanism may have contributed, at least in part, to the benefits observed in the present study (Fan *et al.* 2012). In support, sleep-disordered breathing with its recurrent apnoeas and associated intermittent hypoxia has been shown to stimulate free radical formation via differential regulation of hypoxia-inducible factors 1 and 2, effects that can be reversed through antioxidant prophylaxis (Prabhakar & Semenza, 2012). Furthermore, NO donors have been shown to increase CBF and CVR_{CO_2}, and improve breathing stability (Toda *et al.* 2009). Such benefits are not merely confined to the brain but also the lungs, with acetazolamide known to reduce hypoxic pulmonary vasoconstriction (independently of CA inhibition) and corresponding susceptibility to high-altitude pulmonary oedema (Hohne *et al.* 2007), a syndrome that has equally been associated with a free radical-mediated reduction in pulmonary NO bioavailability (Bailey *et al.* 2010). The recent observation that antioxidant supplementation reversed the inhibitory effects of acetazolamide on the hypoxic ventilatory response (Teppema *et al.* 2006) merely emphasises the complexity of the mechanisms underlying the redox regulation of carotid body function, highlighting the need for further research.

Given its capacity to improve neurovascular function at high altitude, albeit at high doses, it is surprising that acetazolamide's potential as a targeted scavenger of HO• has not been subject to investigation. Classical steady-state competition kinetics experiments using electron paramagnetic resonance spin-trapping techniques will establish its second-order rate constant against HO• formed *in vitro* through 'upstream' photolysis and 'downstream' Fenton chemistry. What features of the molecule ranging from its aromaticity or side substituents in the form of carbonyl and sulfonamide groups contribute to its redox potential also remains to be explored. Finally, it would be of interest to determine whether infusion decreases oxidative stress *in vivo* given the proposed link between HO•-catalysed lipid peroxidation and symptoms associated with high-altitude maladaptation (Bailey *et al.* 2009). These complementary studies ideally performed with a non-CA-inhibiting acetazolamide analogue will help establish if there is indeed a redox basis underlying the neuroprotective benefits of acetazolamide that are secondary to classical CA inhibition.

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